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Population size and habitat quality affect genetic diversity and fitness in the clonal herb, *Cirsium dissectum*

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Abstract Remaining populations of plant species in fragmented landscapes are threatened by declining habitat quality and reduced genetic diversity, but the interactions of these major factors are rarely studied together for species conservation. The interactions between population size, habitat quality, genetic diversity and fitness were investigated in 22 populations of the clonal herb, *Cirsium dissectum* throughout the British Isles. Regression analysis was used to identify significant factors and a structural equation model was developed to illustrate and integrate these interactions. Smaller populations (measured as the total number of plants) had lower genetic diversity (proportion of polymorphic loci), and reduced genetic diversity (allelic richness) had a negative impact on the survival of seedlings grown under standard conditions. Habitat quality also had a large effect on *C. dissectum.* Unmanaged sites with tall vegetation, no bare soil and higher nutrient levels had smaller populations of *C. dissectum,* but flowering was promoted. Flowering was suppressed in heavily grazed sites with short vegetation. Higher levels of bare soil and phosphorus both had a positive influence on genetic diversity, but through distinctly different pathways: bare soil provides safe sites for establishment, whilst phosphorus may promote flowering and improve seed germination. In order to conserve *C. dissectum,* management needs to maintain site heterogeneity so that *C. dissectum* can flower and establishment gaps are still available for seedlings; when either component is reduced, negative feedbacks through reduced genetic diversity and individual fitness can be expected. This study therefore highlights the importance of considering both conservation genetics and habitat quality in the conservation of plant species.

Keywords Plant species conservation, ecological genetics, habitat management, structural equation model

Introduction

Habitat destruction and habitat fragmentation continue to threaten the survival of many species worldwide (Tilman et al. 1994; Hanski and Ovaskainen 2000). Biodiversity research that attempts to understand the processes that occur as species decline is thus very important. Ouborg et al. (2006) define two paradigms within biodiversity research: conservation genetics and habitat quality, and they state that

research tends to concentrate on one element or the other. However, in order to fully understand and develop conservation solutions, habitat quality and genetics need to be considered together.

Conservation genetics emphasises that reductions in population size and increases in population isolation lead to negative consequences that reduce individual fitness and ultimately increase the risk of extinction (Ellstrand and Elam 1993; Reed 2005; Oostermeijer et al. 2003). Genetic diversity is likely to decrease in small populations, as rare alleles are lost (Oostermeijer et al. 2003) and this may reduce the ability of a species to adapt to changing environmental conditions (Barrett and Kohn 1991). Levels of inbreeding can increase as the number of mates available decrease and also through disruption of processes such as plant-pollinator interactions (Oostermeijer et al. 1998). The consequent reduction in heterozygosity can lead to inbreeding depression (Hartl and Clark 1997); in plant species this is often associated with increased seed abortion, low germination rates, high seedling mortality and poor growth and flowering of offspring (Oostermeijer et al. 2003; Dudash and Fenster 2000). Relationships between population size, genetic diversity and fitness have been widely studied and positive correlations between these factors are generally found (Oostermeijer et al. 2003; Leimu *et al* 2006).

Habitat quality, however, may also affect population size, genetic diversity and plant performance by influencing demographic transitions in plant populations. Sexual recruitment, for example, can be reduced or potentially permanently suppressed by environmental variables such as mowing (Schaal and Leverich 1996), canopy closure (Kudoh et al. 1999), climate (Eckert 1999) or an increase in site productivity (Colling et al. 2002; Endels et al. 2004). Reductions in sexual recruitment often lead to a decrease in genetic diversity (Kudoh 1999, Jacquemyn et al. 2005; 2006, Kleijn and Steinger 2002).

In natural populations it is likely that population size, genetic diversity and habitat quality all interact to determine individual fitness and the survival of plant populations (Fig. 1). Studies that take this combined approach are therefore very important but are not frequent within the literature (e.g. Schmidt and Jensen 2000). Oostermeijer et al. (1998) demonstrated that habitat factors play an important role alongside population size and genetic diversity in the performance of the rare species *Gentiana pneumonanthe*. Vergeer et al. (2003) found that larger populations of *Succisa pratensis* had reduced inbreeding and greater fitness, while high soil ammonium had a negative effect on population size and fitness but did not affect genetic diversity.

This study aims to extend this approach to clonal species by exploring key population, habitat and genetic characteristics and relating these factors to individual fitness in *Cirsium dissectum*, an Asteraceae species that shows considerable clonal as well as sexual reproduction.

C. dissectum is found in wet, nutrient-poor, semi-natural grasslands in northwest Europe. It is endangered in Germany and the Netherlands (Buck-Sorlin 1993; Buck-Sorlin and Weeda 2000; Soons et al. 2005) and has declined in all of the countries within which it is found (Institut floristique Franco-Belge 1995; Preston et al. 2002; Hackney 1992).

C. dissectum is quite specialised in its habitat requirements, but can be abundant in suitable conditions; it has declined due to the loss and modification of its habitat (de Vere 2007a). Its sites were traditionally managed through extensive grazing, burning and hay cutting and it is therefore a casualty of the changes in traditional farming practice that have led to losses in all types of semi-natural,

oligotrophic grasslands (HMSO 1995). Reductions in grazing and application of fertilizers have caused an increase in site productivity throughout these grasslands (UK Biodiversity Steering Group 1995). Jongejans et al. (2006a, 2008) discovered that *C. dissectum* is a poor competitor; it is unable to build up biomass, that is necessary to withstand competition, as productivity increases and this reduces the probability of survival.

This study examines the interactions between population size, habitat quality, genetic diversity and individual fitness in a range of natural populations (n = 22) of *C. dissectum* throughout the British Isles and considers the implications of the results for the conservation of *C. dissectum*. Specifically we examine:

a) The effect of management on habitat quality.
b) The effect of population size on genetic diversity and fitness.
c) The effect of genetic diversity on fitness.
d) The effect of habitat quality on population size, genetic diversity and fitness.

Materials and Methods

Study species and sites

Cirsium dissectum (L.) Hill (Asteraceae) is a rhizomatous herb that forms rosettes with up to five softly prickled leaves. Flowering stems are formed apically with normally one flower head (capitulum), rarely two or three. After seed set a rosette dies off. It is self-compatible but selfed plants produce fewer seeds compared with those that are crossed (Kay and John 1994; de Vere 2007b). The species reproduces vegetatively by means of long rhizomes, which then die leaving independent ramets (de Vere 2007b).

Twenty-two populations of *C. dissectum* were selected throughout the species range in the British Isles (Fig. 2). These populations are representative of the range of population sizes and habitat types within which the species is found. For each of these populations the following four groups of measurements were taken: population size, habitat quality, genetic diversity and fitness (these are listed in Table 1).

Population size

Population size is often estimated by counting the number of flowering individuals within a population (e.g. Kéry et al. 2000; Vergeer et al. 2003; Matthies et al. 2004), as this is assumed to provide an estimate of effective population size (Frankham et al. 2002). The number of flowering rosettes was therefore counted for each population during peak flowering time (June). As *C. dissectum* reproduces clonally as well as sexually census population size was also estimated. This was determined as the product of the population area and the density of plants per m^2. Population area was measured using a global positioning system (Garmin eTrex) to provide the latitude, longitude and altitude of plants on the periphery of each population. A software programme written by A. Read (Spirent Communications plc) was then used to connect the points together and measure the area covered. Density was determined by counting the number of flowering and non-flowering rosettes within 30 $1m^2$ quadrats. Populations varied widely in their number of flowering relative to non-flowering rosettes, so the proportion of flowering rosettes in the 30 $1m^2$ quadrats was also calculated and used as an additional variable.

Habitat quality

Five measures of soil nutrients and two measures of vegetation structure were measured. For each of the 22 sites 5 topsoil samples (depth 14cm, diameter 3cm) were taken with an auger. The top and bottom halves of the soil core were separated and the top and bottom samples pooled for the site. Samples were air-dried and stored for later analysis. pH was determined electrometrically after mixing air-dried soil with

distilled water. Organic matter was determined using loss on ignition, total Kjeldahl nitrogen using the Kjeltec system 1002 and extractable phosphorus using Olsen's method (Allen 1989) Calcium was extracted using 1.0M ammonium acetate with lanthanum chloride and potassium with 1M ammonium nitrate, the amounts of these elements was then determined using air-acetylene flame absorption in an atomic absorption spectrophotometer (Varian Spectr AA 50, Varian, UK). The mean vegetation height and percentage cover of bare soil were determined in each of the 30 $1m^2$ quadrats (described above) at each site. Vegetation height was used as an indicator of site productivity; bare soil was measured as this may provide establishment gaps for seedlings. Landowners and managers were asked to describe the management of the site and this resulted in four categories: none, summer grazing (sites grazed for less than six months), continuous grazing (sites grazed for more than six months) or mown.

Genetic diversity

Microsatellite genetic markers were used to determine genetic diversity. Leaf material was sampled from 35 individuals from each of the 22 populations. A systematic sampling strategy was adopted to reduce the possibility of sampling the same clone numerous times. Within each population a transect was established along the longest length of the population, plants were then sampled at either 5 or 10 m intervals depending on the size of population. If by the end of the transect 35 plants had not been sampled then another transect was set up parallel to the first with a 10 m gap. For each plant sampled, a young leaf was removed, cut into 1 cm^2 pieces and placed immediately into silica gel to dry. Samples were stored in dry silica gel until further analysis could take place.

A phenol chloroform extraction based on Doyle and Doyle (1987) was used to obtain high purity DNA. Microsatellite loci were not available for *C. dissectum* but nine loci developed for *Cirsium acaule* by Jump et al. (2002) were re-optimised for use in *C. dissectum;* seven loci amplified consistently and were polymorphic (de Vere 2007a). PCR was performed in a MJ Research PTC-100 thermocycler with a reaction mixture of: 1 µl template DNA (0.250 µg/µl) in a total volume of 10 µl containing 5 µl 2X Thermo-Start PCR Master Mix with 1.5/2.0 mM $MgCl_2$ (ABgene), 0.05 µl DMSO, 1 µl each of forward and reverse primer (5µM) and 1.95 µl ultra high purity water. PCR products were visualised on a CEQ 8000 Genetic Analysis System (Beckman Coulter).

Fitness measures

Three measures of individual fitness were used to characterize plants grown from seed originating from the 22 populations: seed number, germination and seedling survival. Single, ripe, but not dehiscent seed heads were collected from 30 plants from each population and allowed to air-dry. Seed heads were dissected and each seed pressed gently with a pair of forceps to ascertain if it contained an embryo; only those that felt hard were subsequently counted and allowed to germinate.

Seeds were placed in Petri dishes on 3 pieces of Whatman No. 1 filter paper and Petri dishes were then arranged randomly on a heated bench set at 30 ^{o}C. This temperature had previously been found to give the highest germination percentage and rate for *C. dissectum* (de Vere 2007b) . Germination was monitored on a daily basis until no new germination was observed for 2 weeks.

Germinated seeds were removed and placed singly in a 12x7 cell tray filled with seed compost (Coir based tray and modular compost, Goldengrow, UK). Cell trays were arranged randomly on a bench in an unheated glasshouse. When the seedlings had reached a suitable size for potting on, survival was recorded.

Data analysis

GENETIX v.4.02 (Belkhir et al. 2001) was used to determine the proportion of polymorphic loci (P99). Allelic richness and F_{IS} were calculated using FSTAT v2.9.3.2 2002 (Goudet 2001). Duplicate multilocus genotypes, that are likely to represent sampling of the same clone more than once, were removed prior to analysis to ensure that each sample was independent. This resulted in 9.4% of the sampled plants being removed.

For each of the population size, habitat quality and fitness measures, the mean was determined for each population. Top and bottom soil fractions were analysed separately for each of the soil nutrients, so that any structuring of nutrients within the soil profile could be seen. In many cases, however, it was expected that the top and bottom fractions would be highly correlated. Similarly, the different soil nutrients measured were also expected to show some correlations. Bivariate correlations were determined between all soil variables. If the top and bottom soil fraction had a correlation (r) of greater than 0.7, then the mean of the top and bottom was determined and used in subsequent analyses to avoid multicollinearity (Tabachnick and Fidell 2007). In this way, means were used for all soil variables except phosphorus. Correlations were also high for organic matter and total Kjeldahl nitrogen ($r = 0.965$) and for calcium and pH ($r = 0.737$). Therefore nitrogen and calcium were removed from the multivariate analyses. Bivariate correlations were also checked between the other groups of variables, these were all below 0.7.

In order to obtain normality and homogeneity of variance within the data, the total number of rosettes, the number of flowering and the proportion of flowering rosettes, organic matter %, potassium mg kg^{-1}, and phosphorus (7-14 cm) mg kg^{-1} were log-transformed and the proportion of polymorphic loci was arcsine-transformed prior to statistical analysis.

To examine the relationship between management type and habitat quality one-way ANOVAs with post-hoc Tukey tests were performed. Multiple regression analysis using forward, stepwise selection of variables was used to examine the effects of the other variables. The following groups of analyses were carried out:

a) The effect of population size on genetic diversity and fitness. (Response variables: proportion of polymorphic loci; allelic richness; inbreeding coefficient; mean seed number; mean % germination; mean % seedling survival. Explanatory variables: total number of rosettes; number of flowering rosettes; proportion of flowering rosettes).
b) The effect of genetic diversity on fitness. (Response variables: mean % seedling survival; mean seed number; mean % germination. Explanatory variables: proportion of polymorphic loci; allelic richness; inbreeding coefficient).
c) The effect of habitat quality on population size, genetic diversity and fitness. (Response variables: total number of rosettes; number of flowering rosettes; proportion of flowering rosettes; proportion of polymorphic loci; allelic richness; inbreeding coefficient; mean seed number; mean % germination; mean seedling survival. Explanatory variables: vegetation height; bare soil; phosphorus 0-7 cm; phosphorus 7-14 cm; organic matter; potassium; pH).

The interactions between all of these factors were then investigated using structural equation modelling using the AMOS 6 software. A model was constructed that included all significant relationships shown by the multiple regression analyses, along with any correlations between the variables. Maximum likelihood estimation was used to determine the standardised path coefficients; these are equivalent to standardised partial regression coefficients. Model fit to the data was tested using the

likelihood chi-squared value, which tests the null hypothesis that the covariance matrix implied by the model reproduces the observed covariance matrix and Bentler's comparative fit index (CFI) was calculated. Values greater than 0.9 indicate an acceptable fit between the model and the data (Byrne 2001; Iriondo et al. 2003; Grace 2006). Once the model was constructed, we compared it with similar models that included additional relationships that might be expected between the variables on biological grounds to see whether they produced statistically significant standardised path co-efficients.

Results

The total number of rosettes in the 22 sampled populations varied from approximately 3 thousand up to just over 1 million (Table 1). Large variation was seen in the proportion of plants that flowered within a population, ranging from 0 up to 23%. Seeds collected from different populations varied in their percentage germination and seedling survival when grown under standard conditions in the glasshouse. Germination levels were generally low (8.6% ± 8.8% SD), indeed lower than expected for this species (de Vere 2007b).

Phosphorus levels were low throughout all of the sites surveyed, whilst there was more variation in levels of potassium, calcium and organic matter, pH varied from acidic (minimum 4.5) to slightly acidic (maximum 6.1). Sites showed variation in vegetation height from 107 to 833 mm, whilst the amount of bare soil varied from 0 to 20%.

Levels of genetic diversity (both the proportion of polymorphic loci and allelic richness) was variable between populations. The positive inbreeding coefficients (F_{IS}) seen in *C. dissectum* suggests that a certain amount of inbreeding occurs, either through mating between close relatives or selfing (Lowe et al. 2004). The fact that inbreeding levels do not relate to any of the measures of population size suggests that *C. dissectum* is at least partially self-compatible (de Vere 2007b).

Relationships between site management and habitat variables

There were significant relationships between site management and the habitat variables measured within this study (Fig. 3). Sites that were subject to mowing had less potassium, higher calcium and higher pH compared to some of the grazed sites. As the level of grazing intensity increased from none to summer to continuous, the amount of bare soil increased and vegetation height and organic matter decreased. There were also marginally significant trends showing decreased phosphorus (7-14 cm) and nitrogen as grazing intensity increased.

Interactions between population size, habitat quality, genetic diversity, and fitness

The structural equation model (Fig. 4) containing all significant relationships found in the multiple regression analyses (Table 2) showed a good fit between the model and the data; this was indicated by a chi-squared of 42.13; df 46; *P* 0.635 and a CFI of 1.

Population size, measured as the total number of rosettes, had a significant positive relationship with one of the measures of genetic diversity (proportion of polymorphic loci). Another measure of genetic diversity (allelic richness) had a significant positive relationship with one measure of plant fitness (seedling survival).

Of the seven habitat variables included in the analysis, four showed significant relationships with population size, genetic diversity and fitness. Three of these variables were also significantly correlated with each other; vegetation height was positively correlated with phosphorus levels and both were negatively correlated with bare soil. Greater numbers of *C. dissectum* rosettes were associated with sites with shorter vegetation and lower pH; sites with shorter vegetation, however, had

proportionally less rosettes in flower, and the more bare soil, the fewer the total number of flowering rosettes. Sites with tall vegetation and little bare soil therefore have fewer rosettes but those rosettes are more likely to be in flower.

Habitat quality and genetic diversity also revealed significant relationships: sites with more bare soil had *C. dissectum* populations with greater allelic richness. Sites with more phosphorus (7–14 cm) also had greater allelic richness and reduced levels of inbreeding. Finally, habitat quality was related to fitness as sites with more phosphorus showed greater germination of *C. dissectum* seeds under standard conditions.

No other significant standardised path coefficients between the variables were revealed by the additional models. Possible relationships that were tested included whether phosphorus levels were related to any of the measures of population size and whether any of the measures of population size were related to the inbreeding coefficient.

Discussion

Relationships between population size, genetic diversity and fitness

Leimu et al. (2006) found that relationships between population size, genetic diversity and fitness were generally found in a meta-analysis of studies published between 1987 and 2005. Our results are broadly consistent with that picture: we found significantly positive relationships between population size (measured as the total number of rosettes) and genetic diversity, but this only related to one measure of fitness (seedling survival). Population size is often measured as the number of flowering rosettes (e.g. Kéry et al. 2000; Vergeer et al. 2003; Matthies et al. 2004) but in this case none of the measures of flowering related to genetic diversity. Seedling recruitment is very low in *C. dissectum* (Kay and John 1994; Jongejans et al. 2006b, 2008) so the number of flowers in a single year may be a poor estimator of effective population size in this clonal species.

The relationship between genetic diversity and seedling survival may suggest that populations with lower genetic diversity produce seeds that are less able to adapt to the environmental conditions provided in the glasshouse. Growing plants in glasshouse conditions, where all of their requirements are provided, may not be expected to place strong selection pressures on the plants. However, seedling survival was relatively low, so it is possible that plants may have been under some selection pressure with those coming from populations with higher allelic richness better able to survive. Alternatively, maternal effects could explain the relationship between allelic richness and seedling survival. Mother plants found in more suitable environments may have greater allelic richness and these environments may also improve the survival chances of the seedlings.

Interactions between habitat quality, population size, genetic diversity and fitness

Site management effects habitat quality and habitat quality subsequently relates to population size, genetic diversity and fitness in *C. dissectum.* Sites with greater grazing intensities had shorter vegetation and abundant bare soil. These sites tended to have more *C. dissectum* rosettes but fewer of those rosettes flowered. There are a number of possible reasons for this. Ross (1999) showed that experimentally defoliated *C. dissectum* plants produced more ramets than un-defoliated individuals. The larger number of rosettes in sites with short vegetation may therefore be due to increased levels of clonal growth, due to greater grazing intensity. Grazing may also reduce the proportion of rosettes that flower. Bullock et al. (1994) showed that winter grazing of sites containing *Cirsium vulgare* increased the survival of smaller rosettes in the population and thus decreased the proportion of rosettes flowering. The reduced

flowering in sites with short vegetation may also reflect lower productivity as Jongejans et al. (2008) found that more productive sites (estimated as the biomass of clipped vegetation) had a greater proportion of flowering rosettes in grasslands in the Netherlands. They also found that adding fertiliser to *C. dissectum* in experimental conditions increased flowering due to increased rosette growth which resulted in a higher proportion of rosettes reaching the threshold size for flowering.

This study shows an influence of habitat variables on genetic diversity in *C. dissectum* with phosphorus (7–14 cm) and bare soil showing significant positive relationships with levels of allelic richness. Both these factors may lead to increased genetic diversity through increasing levels of successful sexual reproduction. Bare soil is important as it provides establishment gaps for seedlings. A number of studies on other species have shown increased seedling recruitment when the amount of bare soil is greater (e.g Bullock et al. 1994, Hegland et al. 2001, Lennartsson and Oostermeijer 2001), or conversely a reduction in seedling recruitment in more productive sites (Colling et al. 2002; Endels et al. 2004; Soons et al. 2005). This is also likely to be occurring in *C. dissectum.* Seedling recruitment is a "bottleneck" in the population dynamics of this species (Kay and John 1994; Jongejans et al. 2006b, 2008) and establishment of seedlings is promoted by disturbance, such as sod-cutting, that creates bare soil (Isselstein et al. 2002; Jongejans et al. 2006b, 2008). This suggests that sites that do not have bare soil will show a loss of genetic diversity over time, as long-lived clones gradually die and are not replaced with new sexual recruits. It is generally considered that levels of sexual recruitment in clonal plants do not have to be high in order to maintain genetic diversity (Watkinson and Powell 1993; Stehlik and Holdregger 2000). However, if sexual recruitment is very low or non-existent, reductions in genetic diversity are to be expected: other studies have shown reductions in genetic diversity in clonal plants where sexual recruitment is suppressed (Kudoh et al. 1999; Jacquemyn et al. 2005; 2006; Kleijn and Steinger 2002).

The relationship between phosphorus and genetic diversity may be due to a number of mechanisms. Jongejans et al. (2008) has shown that adding nutrients to *C. dissectum* increased flowering. This could be a mechanism leading to increased genetic diversity if greater flowering results in increased successful sexual reproduction. The reduction in the inbreeding coefficient in sites with higher phosphorus supports this hypothesis, as more flowering can increase the number of mates available and may promote greater pollination (Oostermeijer et al. 1998; Frankham et al. 2002). However, our analysis (Fig. 4) shows no relationship between phosphorus levels and flowering, although there seems to be an indirect effect through vegetation height. In addition the number of flowers is unlikely to be a good measure of sexual reproduction due to low seedling recruitment (Kay and John 1994; Jongejans et al. 2006b, 2008).

Phosphorus may promote sexual reproduction by increasing the survival of seedlings, as sites with more phosphorus had seeds that germinated better under standard conditions. The greater germination observed may well be due to better nutrition in the mother plants. However, again this does not necessarily relate to successful sexual reproduction as seeds may be unable to establish in sites with greater productivity due to a lack of safe sites for establishment.

Phosphorus and bare soil may also promote the maintenance of genetic diversity by increasing the survival chances of clonal offspring. Again this mechanism is unlikely for phosphorus, as *C. dissectum* is likely to be out-competed as nutrient levels increase. Bare soil however, may be important for the establishment of clonal

offspring ensuring the long-term survival of clones, and the greater number of rosettes in sites with more bare soil supports this suggestion.

Sites with more bare soil may therefore have *C. dissectum* populations with greater genetic diversity due to increased successful sexual and clonal reproduction; sexual reproduction introduces new genotypes into the population, whilst clonal propagation ensures their long-term survival. The effect of phosphorus on genetic diversity appears more indirect, but would appear to relate to increased sexual reproduction over time, possibly through increased flowering and/or germination that allows the maintenance of genetic diversity. These findings strongly suggest that genetic diversity can be promoted by distinctly contrasting pathways.

Ultimately, the correlative approach taken here has the disadvantage that definite mechanisms cannot be provided for the relationships found. The approach is valuable however, in that it provides hypotheses that can subsequently be tested experimentally. It is also very valuable in that, by taking into consideration a number of the interacting factors, it is now possible to make more comprehensive management recommendations, in order to conserve the species.

Conservation implications

Small populations of *C. dissectum* have less genetic diversity and reduced genetic diversity affects some measures of fitness and subsequently the viability of populations. The protection of existing large populations of *C. dissectum* and the expansion of smaller populations is therefore an important recommendation for the conservation of this species. Unmanaged sites with tall vegetation and no bare soil are likely to have abundant flowering of *C. dissectum* rosettes. Lack of bare soil, however, is likely to prevent the establishment of seedlings and this suppression of sexual recruitment may cause a loss of genetic diversity that can decrease plant fitness and long-term population survival probabilities. Furthermore, *C. dissectum* is unable to build up biomass rapidly in sites with high nutrient levels and will eventually become out-competed (Jongejans et al. 2008). This illustrates that counting the number of flowering rosettes will not provide a good indication of the health of the population.

Conversely, sites that are continually grazed have shorter vegetation, more bare soil and some depletion in nutrients. These sites often have more *C. dissectum* rosettes but flowering is reduced. If flowering is completely suppressed, then sexual reproduction will not be able to occur and genetic diversity will gradually decline.

Site management therefore needs to maintain habitat heterogeneity, so that some flowering can occur, levels of phosphorus are not depleted, and areas of bare soil are maintained to allow for successful recruitment of seedlings and clonal offspring. It is important to note, however, that phosphorus levels are very low in *C. dissectum* sites, the mean is only 3.7 mg kg^{-1} at 0 to 7 cm soil depth and 1.4 mg kg^{-1} at 7 to 14 cm, values typical of semi-natural, species rich grasslands (Goodwin 1995; Tallowin and Smith 2001). If phosphorus levels become too high, then *C. dissectum* is likely to be out-competed by other plant species.

This study highlights the complex interactions occurring between genetics and habitat within the clonal, *C. dissectum*. More studies are required in order to examine the presence of common themes within plant species. A full understanding of the effect of habitat destruction and modification on plant species can only be gained by investigating all of the processes acting upon wild populations simultaneously.

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Table 1. Summary of the variables measured in 22 populations of *Cirsium dissectum* throughout the British Isles. Population means with standard deviations (SD) in parentheses are given, along with the minimum and maximum values recorded. Variables marked with an asterisk were removed from the multivariate analysis to avoid multicollinearity.

	Variable	Mean (SD)	Minimum	Maximum
Population size	Number of flowering rosettes	1308 (1816)	19	5500
	Total number of rosettes	178000 (305000)	3000	1180000
	Proportion of flowering rosettes (%)	4.7 (5.3)	0.0	23.0
Habitat	Vegetation height (mm)	364 (179)	107	833
	Bare soil (%)	7 (7)	0	20
	Phosphorus (P) 0-7cm (mg kg^{-1})	3.7 (4.1)	0.0	12.4
	Phosphorus (P) 7-14cm (mg kg^{-1})	1.4 (1.1)	0.5	5.1
	Organic matter (%)	31 (27)	6	87
	*Nitrogen (N) (%)	0.7 (0.6)	0.1	2.4
	Potassium (K) 0 – 14cm (mg kg^{-1})	117 (109)	18	529
	*Calcium (Ca) 0 – 14cm (mg kg^{-1})	3185 (3583)	248	12112
	pH	5.2 (0.6)	4.5	6.1
Genetic diversity	Proportion of polymorphic loci	0.87 (0.15)	0.43	1.00
	Allelic richness	2.49 (0.39)	1.43	3.04
	Inbreeding coefficient	0.19 (0.08)	0.01	0.36
Fitness	Seed number	33.6 (19.4)	6.5	82.7
	Germination (%)	8.6 (8.8)	0.0	29.3
	Seedling survival (%)	27.1 (22.6)	0.0	69.2

Table 2. Multiple regression analysis with stepwise selection of variables. Three groups of analyses are shown: the effect of population size on genetic diversity and fitness; the effect of genetic diversity on fitness and the effect of habitat quality on population size, genetic diversity and fitness. For each group of analyses the dependant variables (y) are shown in bold and the independent variables (x) in non-bold text. Only significant results are shown. * = *P*-value of < 0.05, ** = *P*-value of < 0.01, *** = *P*-value of < 0.001.

	Beta	***t***	***P***
EFFECT OF POPULATION SIZE ON GENETIC DIVERSITY AND FITNESS			
Proportion of polymorphic loci (R^2 = 0.323*)**			
- Total number of rosettes	0.568	3.088	0.006
EFFECT OF GENETIC DIVERSITY ON FITNESS			
Mean seedling survival (R^2 = 0.270*)			
- Allelic richness	0.519	2.431	0.027
EFFECT OF HABITAT QUALITY ON POPULATION SIZE, GENETIC DIVERSITY AND FITNESS			
Total number of rosettes (R^2 = 0.497)**			
- Vegetation height	- 0.637	- 3.862	0.001
- pH	- 0.423	- 2.566	0.019
Number of flowering rosettes (R^2 = 0.269*)			
- Bare soil	- 0.519	- 2.716	0.013
Proportion of flowering rosettes (R^2 = 0.300)**			
- Vegetation height	0.548	2.930	0.008
Allelic richness (R^2 = 0.546)**			
- Bare soil	0.824	4.666	< 0.001
- Phosphorus 7 – 14 cm	0.560	3.173	0.005
Inbreeding coefficient (R^2 = 0.287)**			
- Phosphorus 7 – 14 cm	- 0.536	- 2.837	0.010
Mean % germination (R^2 = 0.582)**			
- Phosphorus 7 – 14 cm	0.763	5.272	< 0.001

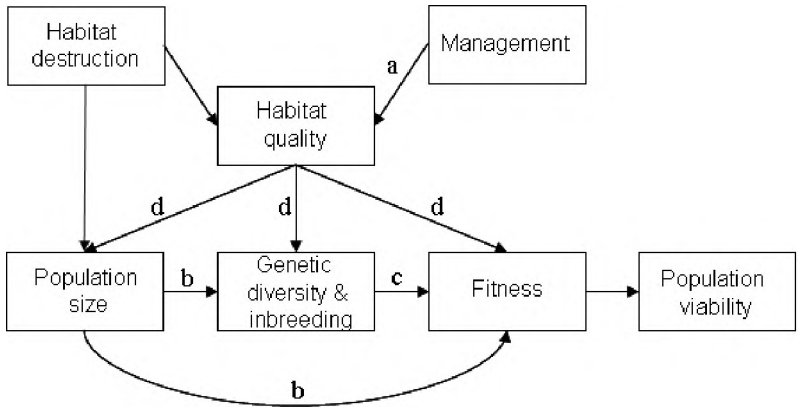

Figure 1. Theoretical relationships between habitat quality, population size, genetic diversity and fitness in plant species. In this paper we investigate: a) the effect of management on habitat quality; b) the effect of population size on genetic diversity and fitness; c) the effect of genetic diversity on fitness and d) the effect of habitat quality on population size, genetic diversity and fitness).

Figure 2. Map of the British Isles showing the locations of the 22 sites containing *Cirsium dissectum* used in this study.

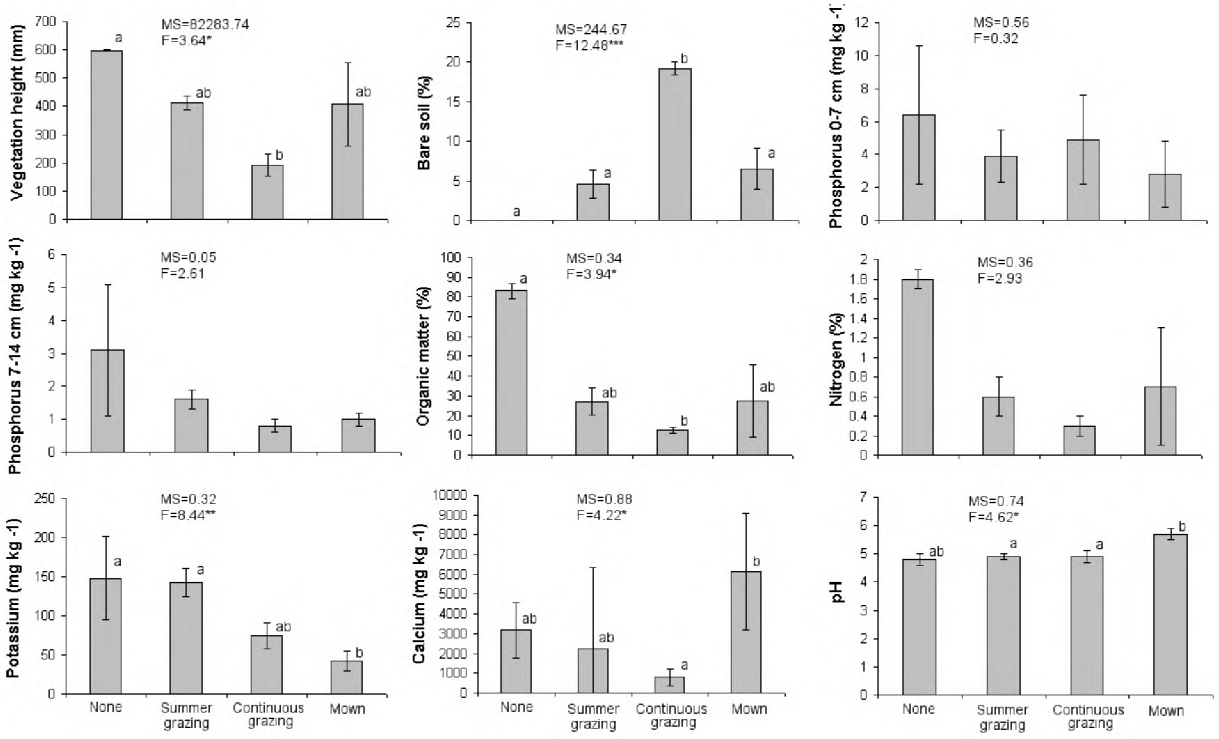

Figure 3. Means with standard error bars for vegetation and soil nutrient values under different site management regimes: none; summer grazing; continuous grazing or mown. Results of a one-way ANOVA are shown (MS = mean square, * = *P*-value of < 0.05, ** = *P*-value of < 0.01, *** = *P*-value of < 0.001). *Post-hoc* Tukey tests were used; sites that do not share a letter are significantly different.

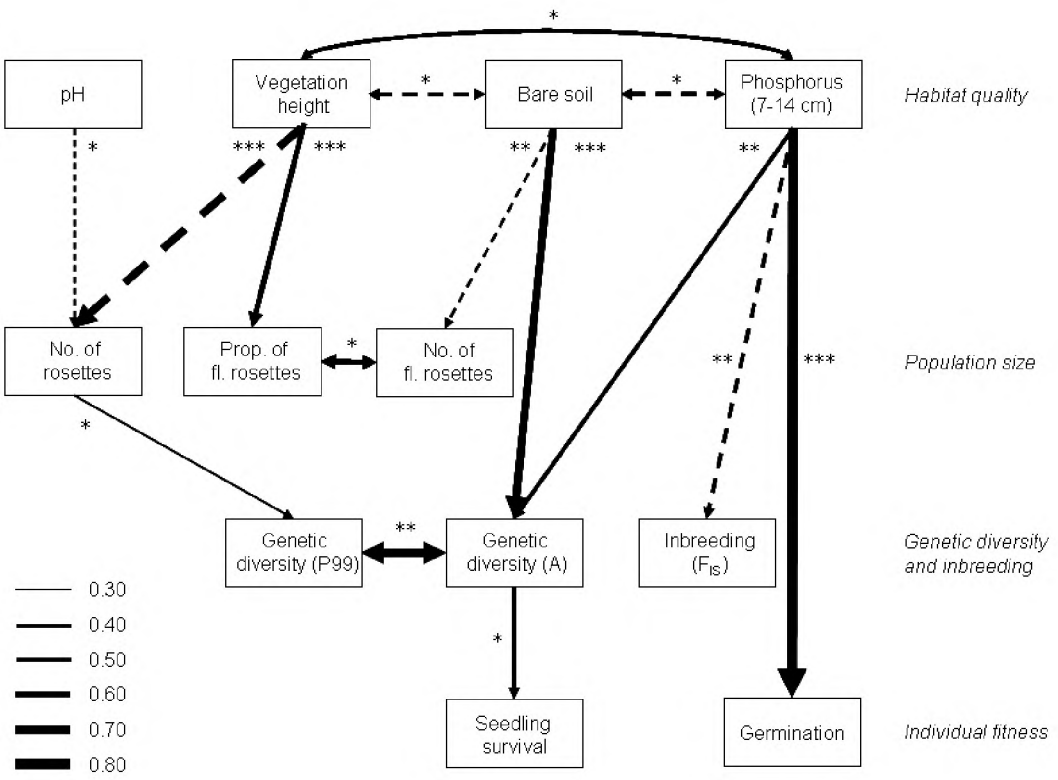

Figure 4. A structural equation model illustrating the interactions between population size, genetic diversity, fitness and habitat quality in *Cirsium dissectum* constructed using the relationships determined during multiple regression analyses. Single headed arrows indicate directional relationships between variables and double-headed arrows show correlations between unmeasured residual variance between variables. The width of each arrow is proportional to the standardised path coefficient, with solid lines indicating positive relationships and dashed lines negative. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The chi-squared statistic is 42.128, df = 46, $P = 0.635$, CFI = 1.00, indicating a good fit between the model and the data.